

Amendments to the claims

1. (withdrawn) A method for obtaining a mammalian cell line adapted to growth in a serum and protein free media, which comprises two stages:

I. A first stage wherein the cell line viability is between 80 and 100% and cells are grown in culture media with consecutive protein concentration reduction up to a critical protein concentration at which cell viability drop to 0%;

II. A second stage wherein once the critical concentration has been predetermined, then the pre-critical concentration is fixed as such protein concentration in which cellular growth is possible and it is the start point to slowly reduce the protein concentration up to the cell culture reaches the initial cellular viability and population doubling time.

2. (withdrawn) The method according to claim 1 wherein the first stage comprises the following steps:

i. Seed 3 wells in the six-well culture plate with recombinant cell line using the standard cell culture medium (with the initial protein concentration). The cell density should be in the range of 1 to 5×10^5 cells/mL. After 48 hours a half of the supernatant is replaced by fresh protein-free medium, thus rendering a final protein concentration which is 50% of the starting condition;

ii. Each 48 hours the supernatant is completely replaced by fresh culture medium with a protein concentration which is 50% of the starting condition;

iii The cells are grown to confluence under this protein concentration;

iv. Cells from step iii are seeded in at least 3 wells at a density in the range 1 to 5×10^5 cells/mL in culture medium with a protein concentration which is 50% of the starting condition. After 48 hours a half of the supernatant is replaced by fresh protein-free medium, rendering a final protein concentration which is 50% of the former condition;

v. Each 48 hours the supernatant is completely replaced by fresh culture medium with a protein concentration which is 50% of the former condition;

vi. The cells are grown to confluence under this protein concentration;

vii. Steps from (iv) to (vi) are repeated, during each cycle the protein concentration is reduced to 50% of the concentration of the previous cycle. This procedure is repeated up to reach a protein concentration which causes cell death.

3. (withdrawn) The method according to claim 1 wherein the second stage comprises the following steps:

viii. Cells are seeded from a cell culture with a viability of 80% or higher growing in the pre-critical protein concentration in at least 3 wells at a density in a range of 2 to 6×10^5 cells/mL. Cells are grown in the pre-critical protein concentration and after 48 hours the 25% of the supernatant is replaced by fresh protein-free medium, thus rendered it a final protein concentration which is the 75% of the pre-critical protein concentration;

ix. Each 48 hours the supernatant is completely replaced by fresh culture medium with a protein concentration which is 75% of the pre-critical protein concentration;

x. The cells are grown to confluence under this protein concentration;

xi. Cells from step (x) are seeded in at least 3 wells at a density in the range 2 to 6×10^5 cells/mL in culture medium with a protein concentration which is 75% of the pre-critical protein concentration. After 48 hours the 25% of the supernatant is replaced by fresh protein-free medium, thus rendered it a final protein concentration which is 75% of the concentration of the previous step;

xii. Each 48 hours the supernatant is completely replaced by fresh culture medium with a protein concentration which is 75% of the concentration of the concentration in step (x);

xiii. The cells are grown to confluence under this protein concentration;

xiv. Steps from (xi) to (xiii) are repeated, during each cycle the protein concentration is reduced to 75% of the concentration of the previous cycle, then this procedure is repeated up to reach a protein concentration which does not cause any lost of cell viability and decrease in population doubling time, When the cells are transferred to a medium with lower protein concentration and they are able to growth without any lost of cell viability and decrease in population doubling time before the first subculture, we could consider that cells have reached again the non-critical stage and seed it directly in protein-free medium (0 mg/mL of protein concentration).

4. (withdrawn) The method according to claim 3 wherein the serum and protein-containing medium in which the cells are initially seeded comprises between 5% and 10% of fetal bovine serum.

5. (withdrawn) The method according to claim 4 wherein the mammalian cell line adapted to growth in a serum and protein free media is a myeloma.

6. (withdrawn) The method according to claim 5 wherein the myeloma is the NSO cell line.
7. (withdrawn) The method according to claim 6 wherein said NSO cell line contains a sequence encoding a recombinant polypeptide or a recombinant protein.
8. (withdrawn) The method according to claim 7 wherein the sequence encoding a recombinant polypeptide or a recombinant protein codifies for a recombinant antibody or a fragment thereof.
9. (currently amended) A mammalian NSO cell line obtained by ~~the a method of claim 3, wherein said for obtaining a mammalian cell line is~~ adapted to grow in a serum and protein free media, which comprises the following two stages:
- I. a first stage wherein the cell line viability is between 80 and 100% and cells are grown in culture media with consecutive protein concentration reduction up to a critical protein concentration at which cell viability drops to 0%,
- II. a second stage wherein once the critical concentration has been predetermined, then a pre-critical concentration is fixed as such protein concentration in which cellular growth is possible and said pre-critical concentration is the start point to slowly reduce the protein concentration up to a point where the cell culture reaches an initial cellular viability and population doubling time;

the second stage comprising the following steps:

viii) seeding cells from a cell culture with a viability of 80% or higher growing in the pre-critical protein concentration in at least 3 wells at a density in a range of 2 to 6 x 10⁵ cells/mL;

ix) growing the cells in the pre-critical protein concentration and after 48 hours replacing 25% of the supernatant with a fresh protein-free medium, thus rendering a final protein concentration of 75% of the pre-critical protein concentration;

x) completely replacing each 48 hours the supernatant with a fresh culture medium having a protein concentration which is 75% of the pre-critical protein concentration;

xi) growing the cells to confluence under said protein concentration;

xii) seeding the cells from step (x) in at least 3 wells at a density in the range 2 to 6×10^5 cells/mL in culture medium with a protein concentration which is 75% of the pre-critical protein concentration;

xiii) replacing after 48 hours 25% of the supernatant with a fresh protein-free medium, thus rendering a final protein concentration which is 75% of the concentration of the previous step;

xiv) completely replacing each 48 hours the supernatant with a fresh culture medium with a protein concentration which is 75% of the concentration of the concentration in step (xii);

xv) growing the cells to confluence under said protein concentration;

xvi) repeating steps from (xii) to (xv) wherein during each cycle the protein concentration is reduced to 75% of the concentration of the previous cycle, then the procedure being repeated up to the attainment of a protein concentration which does not cause any loss of cell viability and decrease in population doubling time, when the cells are transferred to a medium with lower protein concentration and they are able to grow without any loss of cell viability and decrease in population doubling time before the first subculture wherein the cells reach the non-critical stage and are seeded directly in protein-free medium (0 mg/mL of protein concentration).

10-11. (canceled)

12. (currently amended) A mammalian cell line according to claim 9 †† wherein the NSO cell line contains a sequence encoding a recombinant polypeptide or a recombinant protein.

13. (previously presented) A mammalian cell line according to claim 12 wherein the sequence encoding a recombinant polypeptide or a recombinant protein codifies for a recombinant antibody or a fragment thereof.

14. (previously presented) A mammalian cell line according to claim 13 wherein the sequence codifies for the humanized recombinant antibody anti-EGF-R hR3 or a fragment thereof.

15. (withdrawn) A mammalian cell line according to claim 13 wherein the sequence codifies for the humanized recombinant anti-CD6 antibody T1hT or a fragment thereof.

16. (withdrawn) A mammalian cell line according to claim 13 wherein the sequence codifies for the chimeric recombinant anti-CD3 antibody T3Q or a fragment thereof.

17. (withdrawn) Use of the method according to claim 3 for obtaining a mammalian cell line adapted to growth in a serum and protein free media.

18. (withdrawn) The humanized monoclonal antibody against EGF-R hR3 or fragments thereof secreted by the cell lines obtained by the method of claim 3.

19. (withdrawn) The humanized monoclonal antibody against CD6 antigen T1hT or fragments thereof secreted by the cell lines obtained by the method of claim 3.

20. (withdrawn) The chimeric monoclonal antibody against CD3 antigen T3Q or fragments thereof secreted by the cell lines obtained by the method of claim 3.